separation and identification capabilities, and is therefore superior to GC alone.

Both GC and GC-MS, although being suitable techniques for the separation and analysis of explosives, have a limitation in that the injector and column have to be heated. This fact necessitates taking special precautions when dealing with the more thermally labile compounds. Liquid chromatography–mass spectrometry (LC-MS), where the injector and column are at room temperature, does not have these limitations, and is therefore a better choice when analysing the more thermally labile explosives. However, GC-MS is readily available in most analytical laboratories, while LC-MS is not. This situation is expected to change in the next 5 to 10 years, which will place LC-MS as the method of choice for the separation and analysis of explosives.

In both GC-MS and LC-MS, the addition of tandem mass spectrometry (MS-MS) provides an extra dimension for improved selectivity and therefore improved identification.

See Colour Plate 83.

See also: **II/Chromatography: Gas:** Detectors: Mass Spectrometry; Detectors: Selective. **Extraction:** Analytical Extractions; Solid-Phase Extraction; Solid-Phase Microextraction. **Explosives:** Liquid Chromatography; Thin-Layer (Planar) Chromatography.

Further Reading

Douse JMF (1987) Improved method for the trace analysis of explosives by silica capillary column gas chromatography with thermal energy analysis detection. *Journal of Chromatography* 410: 181–189.

Douse JMF and Smith RN (1986) Trace analysis of explosives and firearm discharge residues in the Metropolitan Police Forensic Science Laboratory. *Journal of Energetic Materials* 4: 169–186.

Feltes J, Levsen K, Volmer D and Spiekermann M (1990) Gas chromatographic and mass spectrometric determination of nitroaromatics in water. *Journal of Chromatography* 518: 21–40.

Francis ES, Wu M, Farnsworth PB and Lee ML (1995) Supercritical fluid extraction/gas chromatography with thermal desorption modulator interface and nitro-specific detection for the analysis of explosives. *Journal of Microcolumn Separations* 7: 23–28.

Haas R, Schreiber I, v. Low E and Stork G (1990) Conception for the investigation of contaminated munition plants. 2. Investigation of former RDX-plants and filling stations. *Fresenius Journal of Analytical Chemistry* 338: 41–45.

Hable M, Stern C, Asowata C and Williams K (1991) The determination of nitroaromatics and nitramines in ground and drinking water by wide-bore capillary gas chromatography. *Journal of Chromatographic Science* 29: 131–135.

Kolla P (1994) Gas chromatography, liquid chromatography and ion chromatography adapted to the trace analysis of explosives. *Journal of Chromatography A* 674: 309–318.

Slack GC, McNair HM and Wasserzug L (1992) Characterization of Semtex by supercritical fluid extraction and off-line GC-ECD and GC-MS. *Journal of High Resolution Chromatography* 15: 102–104.

Tamiri T, Zitrin S, Abramovich-Bar S, Bamberger Y and Sterling J (1992) *GC/MS Analysis of PETN and NG in Post-Explosion Residues*. In: Yinon J (ed.) *Advances in Analysis and Detection of Explosives*, pp. 323–334. Dordrecht: Kluwer Academic Publishers.

Welsch T and Block H (1997) Separation and enrichment of traces of explosives and their by-products from water by multiple micro liquid extraction for their determination by capillary gas chromatography. *Fresenius Journal of Analytical Chemistry* 357: 904–908.

Yinon J (1996) Trace analysis of explosives in water by gas chromatography–mass spectrometry with a temperature-programmed injector. *Journal of Chromatography A* 742: 205–209.

Yinon J and Zitrin S (1993) *Modern Methods and Applications in Analysis of Explosives*. Chichester: John Wiley.

DropBooks

Liquid Chromatography

U. Lewin-Kretzschmar, J. Efer and W. Engewald, University of Leipzig, Leipzig, Germany

Copyright © 2000 Academic Press

Introduction

Explosive analysis is important in different areas: explosive manufacture (quality and wastewater control), forensic science and toxicology (investigation of explosions or of criminal actions) and environmental monitoring (water and soil analysis at sites intensively used for military purposes). **Figure 1** shows some of the more common explosives.

In the literature various methods and procedures have been described for analysing these compounds. In the last few years, the investigation of explosives in the water and soil around former ammunition plants,

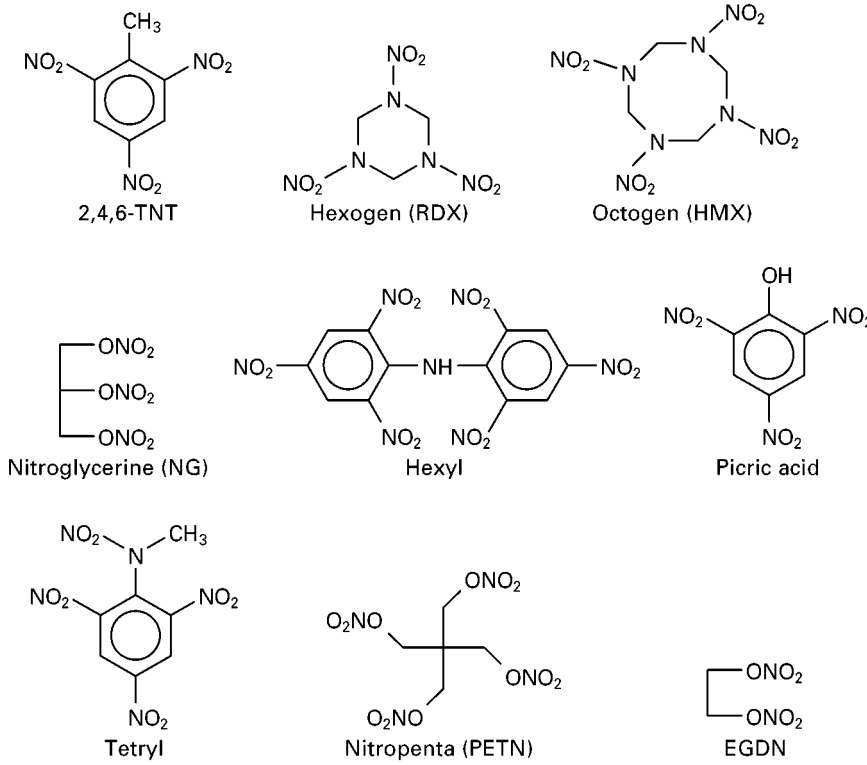


Figure 1 Structures of common explosives.

munition depots or dumps dating back to World War II has become increasingly important. In these samples there are not only explosives but also their by-products and metabolites. For example, water samples from around the former ammunition plant at Elsnig (Saxony, Germany) contained many explosive-related compounds of various classes (**Table 1**).

These constituents, which range in concentrations from ng L^{-1} to mg L^{-1}, make it difficult to analyse such samples. However, to assess the toxic potential, reliable analysis of all the compounds down to the trace amounts (about 0.1 μg L^{-1}) is necessary, as some are highly toxic, carcinogenic or mutagenic.

Capillary gas chromatography offers advantages of separation efficiency and favourable detection limits; however, because of the thermal instability and high polarity of some compounds, high performance liquid chromatography (HPLC) determination is often the method of choice. This requires thorough optimization of HPLC conditions (stationary and mobile-phase) as well as selective and sensitive detection systems and, in some cases, selective sample preparation or pre-separation of the sample into different fractions.

The aim of this article is to provide an overview of the possibilities given by HPLC methods to determine explosive-related compounds in complex samples, including separation, detection and sample preparation, focusing in particular on compounds occurring in samples around former ammunition plants (Table 1).

Sample Preparation

Water Samples

Brown glass bottles should be filled up to the brim with water samples and the bottles made gas-tight, for example, using Teflon packings, and stored at 4°C. To prevent adsorption losses or degradation at the glass surfaces, the addition of methanol or acetonitrile or the use of silanized glass vessels is recommended. To prevent bacterial degradation, sodium azide (about 0.5 g L^{-1}) can be added to the samples.

In principle, for extraction and enrichment, various methods such as liquid–liquid extraction (LLE), solid-phase extraction (SPE) or solid-phase microextraction (SPME) can be used.

Neutral compounds An effective and reproducible liquid–liquid extraction of neutral explosive compounds such as the nitroaromatics can take place with various solvents such as dichloromethane, ethyl

Table 1 Compounds in water samples in the neighbourhood of the former ammunition plant in Elsnig

Compounds	*Abbreviations*
Nitroaromatics and nitramines	
2,4,6-Trinitrotoluene	2,4,6-TNT
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX/Hexogen
2,2',4,4',6,6'-Hexanitrodiphenylamine	Hexyl
1,3-Dinitrobenzene	1,3,-DNB
2,4-Dinitrotoluene	2,4-DNT
2,6-Dinitrotoluene	2,6-DNT
3,4-Dinitrotoluene	3,4-DNT
Nitrobenzene	NB
2-Nitrotoluene	2-NT
3-Nitrotoluene	3-NT
4-Nitrotoluene	4-NT
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	Octogen/HMX
1,3,5-Trinitrobenzene	1,3,5-TNB
Chloroaromatics	
Chlorobenzene	CIB
1-Chloro-2,4-dinitrobenzene	1-Cl-2,4-DNB
1-Chloro-4-nitrobenzene	1-Cl-4-NB
1,2-Dichlorobenzene	1,2-DClB
1,4-Dichlorobenzene	1,4-DClB
2,3-Dichloronitrobenzene	2,3-DClNB
2,5-Dichloronitrobenzene	2,5-DClNB
1,2,4-Trichlorobenzene	1,2,4-TClB
Amino- and aminonitroaromatics	
2-Amino-4,6-dinitrotoluene	2-A-4,6-DNT
4-Amino-2,6-dinitrotoluene	4-A-2,6-DNT
2-Amino-4-nitrotoluene	2-A-4-NT
2-Amino-6-nitrotoluene	2-A-6-NT
4-Amino-2-nitrotoluene	4-A-2-NT
2,6-Diamino-4-nitrotoluene	2,6-DA-4-NT
2,3-Diaminotoluene	2,3-DAT
2,4-Diaminotoluene	2,4-DAT
2,6-Diaminotoluene	2,6-DAT
3,5-Dinitroaniline	3,5-DNA
Nitrophenols	
2,4-Dinitrophenol	2,4-DNP
3,4-Dinitrophenol	3,4-DNP
3,5-Dinitrophenol	3,5-DNP
2-Methyl-4,6-dinitrophenol	2-M-4,6-DNP
4-Methyl-2,6-dinitrophenol	4-M-2,6-DNP
3-Methyl-2-nitrophenol	3-M-2-NP
3-Methyl-4-nitrophenol	3-M-4-NP
4-Methyl-2-nitrophenol	4-M-2-NP
5-Methyl-2-nitrophenol	5-M-2-NP
3-Nitrophenol	3-NP
4-Nitrophenol	4-NP
2,4,6-Trinitrophenol	Picric acid/PA
Nitrobenzoic acids	
2-Amino-4-nitrobenzoic acid	2-A-4-NBA
2,4-Dinitrobenzoic acid	2,4-DNBA
2-Nitrobenzoic acid	2-NBA
3-Nitrobenzoic acid	3-NBA
4-Nitrobenzoic acid	4-NBA

acetate, methyl isobutylketone and methyl tert-butyl ether at different pH values. As a rule, recovery of $>70\%$ can be achieved with triple extraction (stirring or shaking in an Erlenmeyer flask). In order to obtain good recovery for the relatively highly volatile mononitrated aromatics and chloroaromatics, great care should be taken when concentrating or redissolving extracts. To extract polar compounds like hexogen, octogen or nitroguanidine, continuous LLE in rotary perforators is more effective (**Figure 2**).

Good results are also obtained with SPE. For neutral nitroaromatics recoveries of between 70 and 100% are reached with octadecylsiloxane-bonded silica materials (RP-18). Increasingly, the highly porous (specific surface $>1200\ m^2\ g^{-1}$) and high purity adsorbents based on styrene-divinylbenzene (SDVB) copolymer are used and these are particularly suitable for the more water soluble compounds such as nitramines.

The US Environmental Protection Agency (EPA) method makes use of salting-out-effects in the extraction. This enables nitramines, nitroaromatics and nitrate esters to be extracted with solvents freely miscible with water, such as acetonitrile. Here, good recoveries are obtained, but reproducibility is not as good as with the methods mentioned above.

Acidic compounds The extraction of acidic compounds such as nitrophenols and nitrobenzoic acids should take place at low pH in order to ensure the presence of these compounds in their nondissociated form. For this purpose a pH value of 2 has proved successful.

Also, continuous extraction in a rotary extractor leads to a higher yield than is the case in discontinuous extraction (**Figure 3**). Suitable solvents are dichloromethane and ethyl acetate.

In general, lower recoveries will be obtained for the volatile ortho-substituted nitrophenols if a concentration step is necessary after the extraction.

High recoveries are obtained on SDVB copolymers. In SPE with RP-18 materials, recoveries $>70\%$ for the mononitrophenols are only reached if large amounts of salt ($300\ g\ NaCl\ L^{-1}$) are added.

An efficient enrichment of acidic compounds is also possible after ion pair formation with tetrabutylammonium chloride in a neutral to basic medium or by extractive derivatization by means of acetic anhydride or pentafluorobenzoyl chloride in the presence of a phase transfer catalyst with dichloromethane as solvent.

Basic compounds An efficient enrichment of aminoaromatics and especially of diaminoaromatics can be reached at pH 12 with continuous LLE with dichloromethane or SPE on SDVB copolymer materials (**Figure 4**). Discontinuous extraction with

DroidBooks

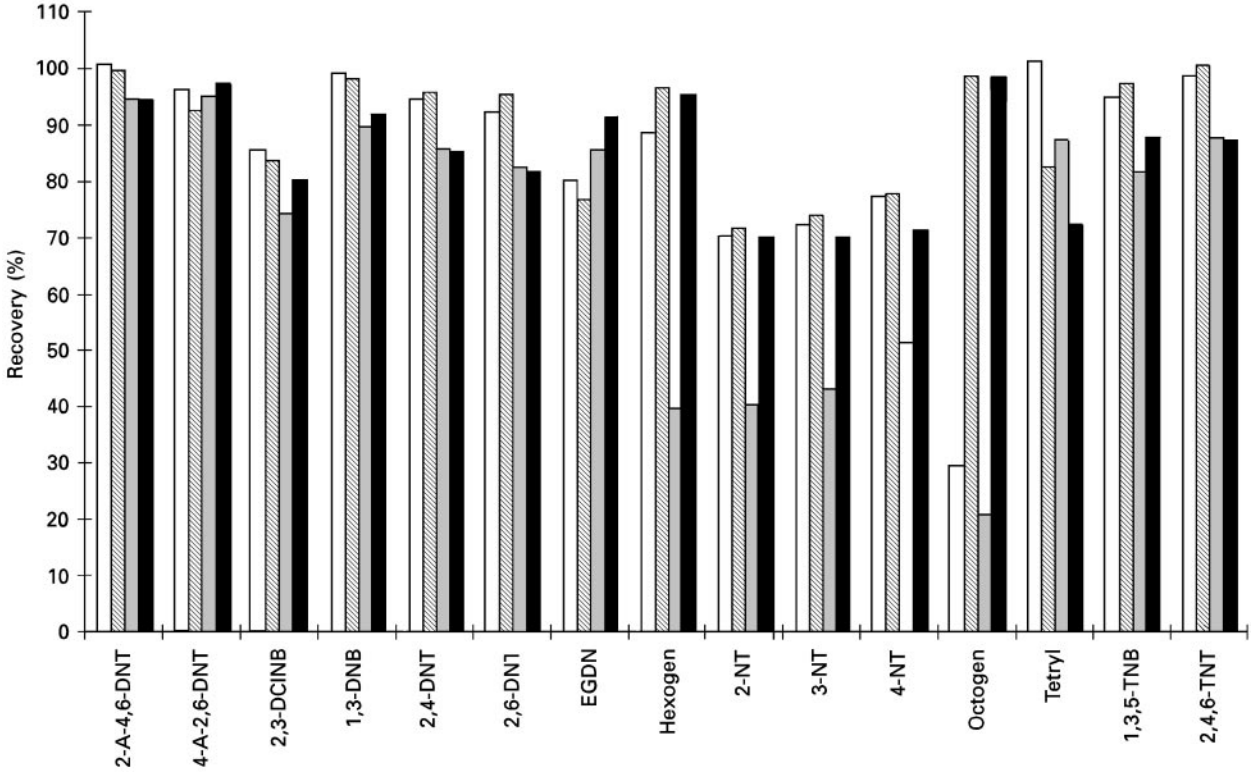


Figure 2 Comparison of various extraction techniques for neutral compounds. Samples of 0.5 L (water spiked with 2 μg L^{-1} for each component and adjusted to pH 9 with 0.1 mol L^{-1} sodium hydroxide were enriched as follows: (A) discontinuous LLE (open columns): stirring three times with 25 mL dichloromethane for 30 min in an Erlenmeyer flask; (B) continuous LLE (hatched columns): 0.5 L extraction with 150 mL dichloromethane in a heavy-phase rotary perforator (Normag); (C) SPE-RP-18 (dotted columns): enriched on 2 g PolarPlus (Baker), conditioned with 3 mL acetone, methanol and water, and eluted with 6 mL methanol; (D) SPE-SDVB (filled columns): enriched on 200 mg LiChrolut EN (Merck), conditioned with 3 mL acetonitrile, methanol and 9 mL water, and eluted with 2 mL methanol acetonitrile mixture (1 : 1).

dichloromethane is not effective. The use of RP-18 materials or ethyl acetate as a solvent is out of the question because of the high pH.

Fractionation Where there are very complex samples, for example, the water samples from Elsnig, precise qualitative and quantitative analysis is only possible after pre-separation of the components. A suitable method is class fractionation based on LLE (**Figure 5**) at various pH values.

Here, by means of the discontinuous dichloromethane extraction at pH 9, the nitro and monoaminoaromatics – which in most cases are the main contaminants – can be almost completely separated from the acidic and basic compounds. Only the more polar nitramines are not completely extracted and are partially included in the other two fractions. Using a more efficient extraction technique in the first extraction step (for example, continuous extraction or SPE on SDVB), selective pre-separation is not possible since acidic and basic compounds would also be partially extracted.

Soil Samples

The basic condition for reliable analysis of soil samples is representive sampling and good homogenization of the samples.

The method most frequently used to prepare the soil samples to analyse explosives is extraction in an ultrasonic bath. Compared with Sohxlet extraction it has several advantages: careful treatment of thermolabile compounds, easy handling, minimum apparatus expenditure and low consumption of solvents.

Suitable solvents are, in principle, acetone, methanol and acetonitrile. However, with a view to the subsequent HPLC determination, methanol and acetonitrile should rank first.

Nitroaromatics are quantitatively extracted by both solvents. But for the more polar compounds like octogen, hexogen and hexyl, acetonitrile is preferred (**Table 2**).

Ultrasonic extraction is described in detail in US-EPA-8330. In this case, 2 g of soil is extracted

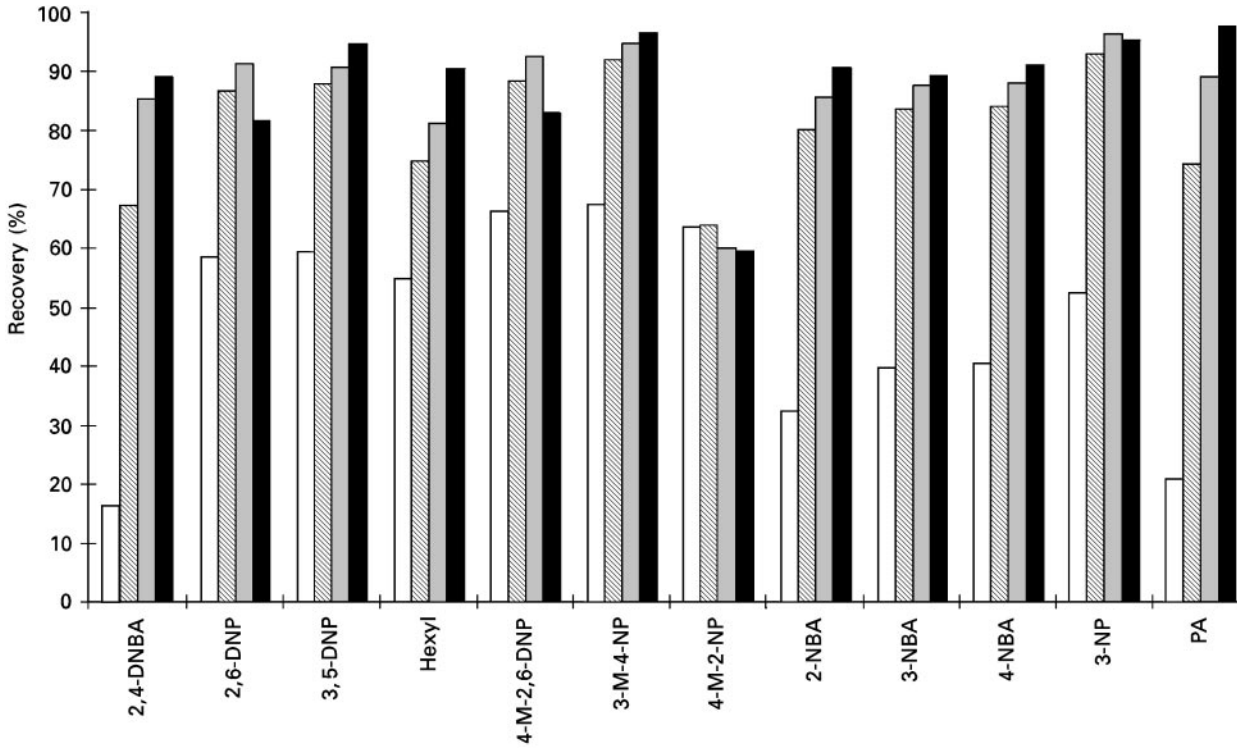


Figure 3 Optimization of extraction for acidic compounds. Samples of 0.5 L (water spiked with 2 μg L^{-1} for each component and adjusted to pH 2 with 0.1 mol L^{-1} hydrochloric acid) were extracted with 150 mL dichloromethane in a heavy-phase rotary preforator or with 200 mL ethyl acetate in a light-phase rotary perforator, respectively. Open columns, MeCl 3 × 30 min; hatched columns, MeCl 4 h; dotted columns, MeCl 10 h; filled columns, Etac 4 h.

DropBooks

with 10 mL acetonitrile over 18 h at room temperature.

A new efficient method for extraction of soil samples is accelerated solvent extraction (ASE), which has proved suitable for nitroaromatics.

HPLC Separation Systems

In general, RP-18 materials are used to separate explosive-related compounds. According to sample composition and detector selection, the composition of the mobile phase (type and amount of organic modifier, buffer additives) varies considerably. The most common mobile phases are buffered or unbuffered methanol–water and acetonitrile–water mixtures in isocratic or gradient operation.

Ethanol, *n*-propanol and dioxane as modifiers or ternary solvent mixtures such as water–methanol–acetonitrile or water–methanol–tetrahydrofuran are of little practical importance and result in too small a selectivity change compared with binary mobile phases.

Because of the generally limited separation performance of HPLC, complete separation of all explosive-related compounds cannot be achieved on any one column in one chromatographic run, not even under carefully optimized separation conditions. Therefore, before separation in the case of complex samples, pre-separation of the components into different fractions by HPLC is useful.

Neutral Compounds

RP-18 phases are very suitable for separation of complex mixtures of nitro- and nitroaminoaromatics, nitramines and nitrate esters with methanol–water or methanol–buffer mobile phases.

In general, at a methanol–water ratio of about 1:1, the retention of compounds on RP-18 phases will increase as follows: nitroguanidine < octogen < hexogen < EGDN < DEGN < 1,3-DNB < 2,4,6-TNT < 4-A-2,6-DNT < 2-A-4,6-DNT < 2,6-DNT < 2,4-DNT < 2-NT < 4-NT < 3-NT < PETN < diphenylamine (**Figure 6**).

For some compounds, however, various RP-18 columns show different selectivities, which result in coelution and retention reversal of various pairs of substances, as shown in **Table 3**. Two columns of complementary selectivity can be used to verify the separation results or to reduce peak overlapping.

If chlorinated aromatics additionally occur in real samples (Table 1) these can be determined under the same conditions as the nitroaromatics. However, for

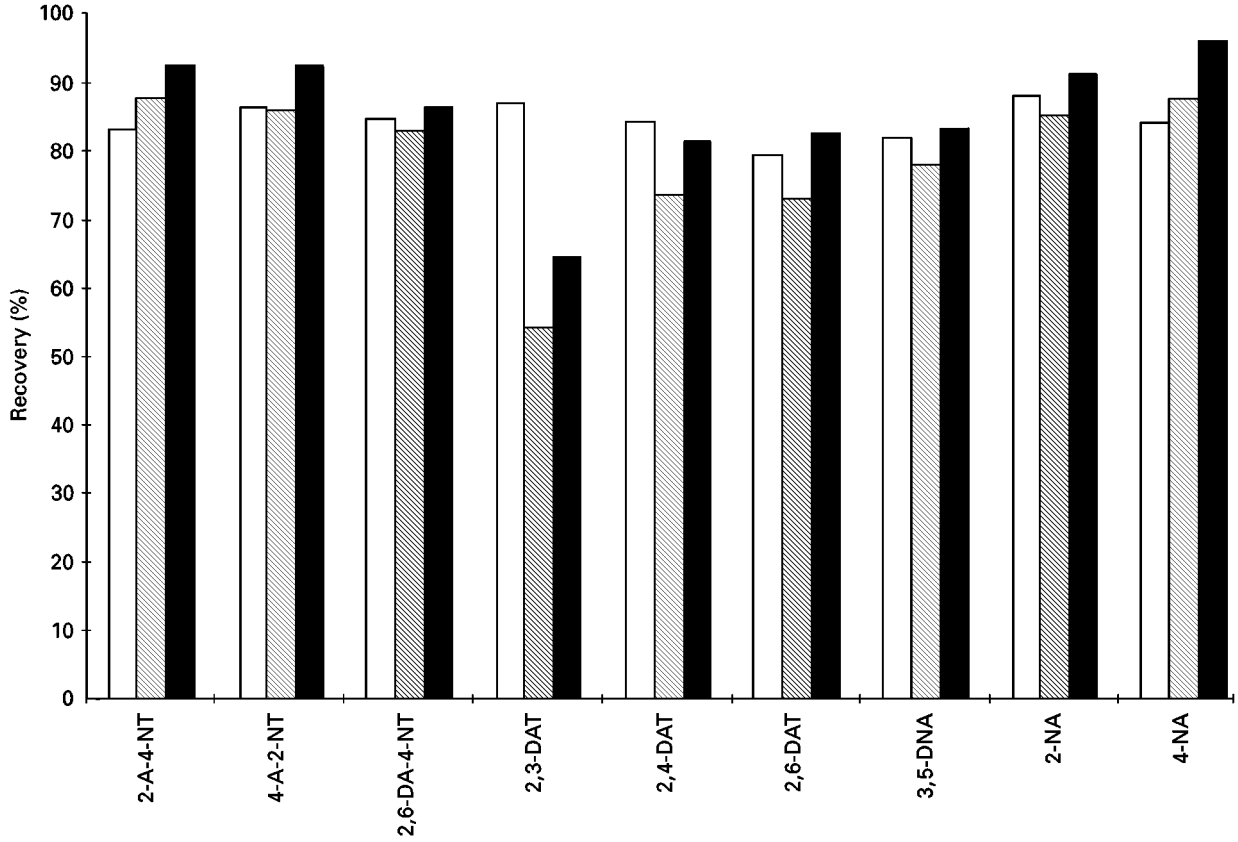


Figure 4 Extraction of basic compounds. Samples of 0.5 L (water spiked with 2 μg L^{-1} for each component and adjusted to pH 12 with 1 mol L^{-1} sodium hydroxide) enriched (A) by continuous LLE with 150 mL dichloromethane and (B) by SPE on 500 mg LiChrolut EN (Merck), conditioned with 3 mL acetonitrile, methanol and 9 mL water, and eluted with a 2 mL methanol/acetonitrile mixture (1:1). Open columns, LLE 4 h; hatched columns, LLE 10 h; filled columns, SPE-SVDB.

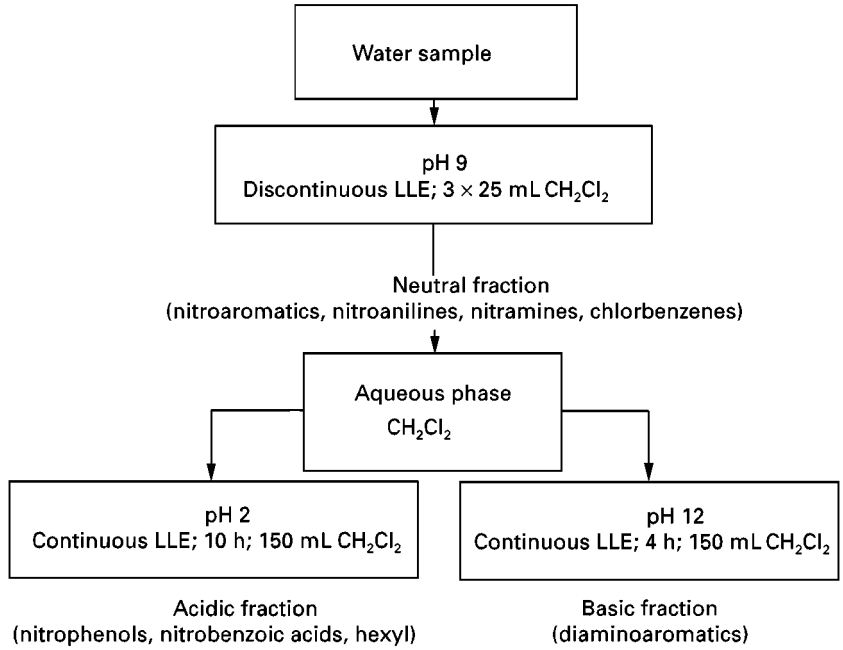


Figure 5 Optimized fractioned extraction procedure. For description of the extraction steps see Figures 2–4. (Reproduced with permission from Lewin U *et al.* (1997) *Chromatographia* 45: 91.)

Table 2 Extraction of spiked soil samples with different solvents

Compound	*Acetonitrile*		*Methanol*	
	Recovery (%)	*RSD (%)*	*Recovery (%)*	*RSD (%)*
Hexogen	97	2.5	82	2.4
Hexyl	86	2.2	73	1.5
Octogen	95	3.4	69	4.3
2,4,6-TNT	98	2.1	98	2.1

2 g soil spiked with 10 mg kg^{-1} for each component, extracted with 10 ml solvent for 15 min in an ultrasonic bath. RSD, relative standard deviation ($n = 3$).

the more retained dichlorocompounds, gradient elution is recommended.

To clarify peak overlapping, other phases in addition to RP-18 phases have proven their value. US-EPA, for example, recommends a cyanopropyl column as a second column showing a clearly different selectivity: nitroguanidine < NB < toluol < 2-NT < 4-N....T < 3-NT < EGDN < 1,3-DNB < 2,6-DNT < 2,4-DNT < TNT < 4-A-2,6-DNT < 2-A-4,6-DNT < hexogen < tetryl < diphenylamine < octogen < PETN.

Similar retention orders are also observed under normal-phase conditions on silica gel, cyanopropylsiloxane and aminopropylsiloxane-bonded silica sorbents. As the normal-phase mode has considerable disadvantages (disturbance by traces of water, no gradient elution), these separation systems are used only rarely and in most cases only with detectors which are incompatible with aqueous mobile phases, such as the thermal energy analyser (TEA) and the electron-capture detector (ECD).

Large selectivity differences in RP-18 phases are also obtained on nitrophenyl-modified silica gel and on porous graphitic carbon (PGC) (**Table 4**).

Thus, retention on these phases will increase with the growing number of nitro groups. Furthermore, in contrast to the RP-18 columns, large retention differences are observed for the isomeric dinitro and aminodinitro compounds. In addition, the PGC phase, due to its high hydrophobicity, shows generally higher retentions, which require a higher methanol content (> 85%) and the separation performance is not satisfactory, due to the low efficiency of such columns.

In addition to the commercially obtainable columns, special materials such as the charge transfer phases like arylpropylether, *N*-propylaniline and safrol phases or a two-dimensional coupling of RP-18 phases with these columns for increasing the selectivity have been tested but offer no great advantage over the common materials.

DropBooks

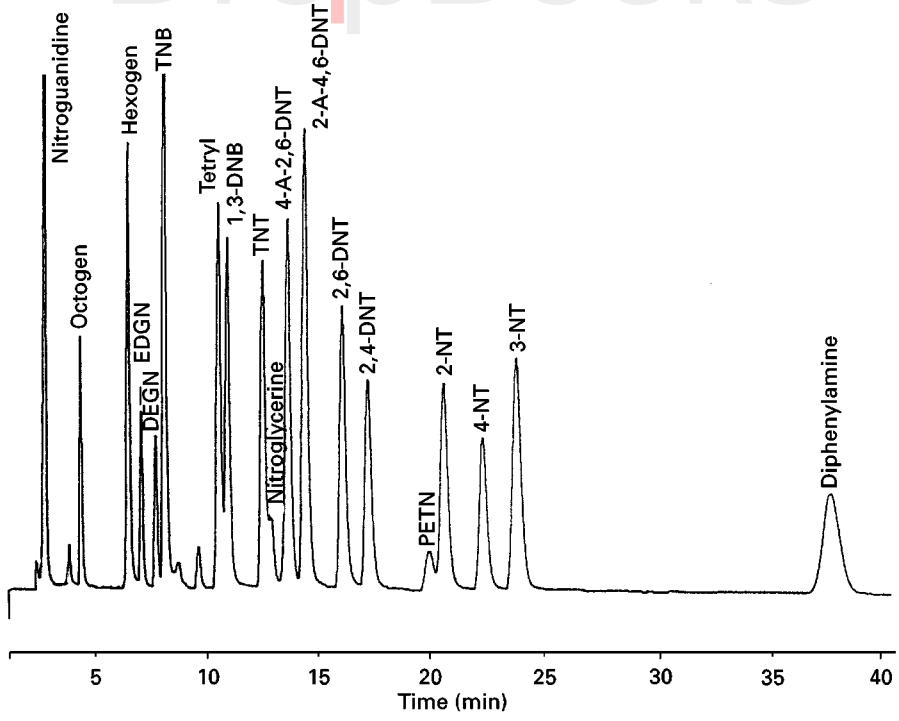


Figure 6 Chromatogram of a standard mixture of explosive-related compounds. 51% methanol–49% water (v/v); Spherisorb ODS 2 column, 5 μm, 250 × 4 mm (HP); 1 mL min^{-1}; 25°C; UV 254 nm.

Table 3 Retention behaviour of neutral explosive-related compounds on different RP-18 columns

Column	*Spherisorb ODS 2 (HP)*		*Eurospher 100 C18 (Knauer)*		*UltraSep Es EX (Sepserv)*	
Compound	*Peak order*	*k*[a]	*Peak order*	*k*[b]	*Peak order*	*k*[c]
NG	1	0.22	1	0.14	1	0.07
Octogen	2	0.60	2	0.63	2	0.51
Hexogen	3	1.55	3	1.67	3	1.82
1,3,5-TNB	4	2.21	5	2.26	4	2.51
2-A-6-NT	5	2.23	4	2.00	5	2.71
2-A-4-NT	6	2.74	6	2.59	6	3.03
Tetryl	7	3.03	8	3.84	7	3.61
1,3-DNB	8	3.48	7	3.47	8	4.37
2,4,6-TNT	9	3.90	11	4.98	10	4.56
NB	10	4.00	9	4.14	8	4.14
4-A-2,6-DNT	11	4.36	12	5.63	11	5.36
3,4-DNT	12	4.68	10	4.36	12	5.41
2-A-4,6-DNT	13	4.72	13	5.85	13	6.18
2,6-DNT	14	5.55	14	6.09	14	6.67
2,4-DNT	15	6.06	15	6.28	15	7.63
2-NT	16	7.67	16	7.60	16	8.48
4-NT	17	8.36	17	8.32	17	9.46
3-NT	18	9.05	18	8.98	18	10.16

Dimension of each column: 250 × 4 mm; 5 μm; [a]hold-up time 1.57 min; [b]hold-up time 1.70 min; [c]hold-up time 1.18 min. 51% methanol–49% water (v/v); 1 ml min^{-1}; 27°C; 10 μg mL^{-1} per component.

Acidic Compounds

The separation of the acidic explosives hexyl and picric acid and the metabolites nitrophenols and nitrobenzoic acids is, in principle, possible on RP-materials under the following conditions: at acidic pH value; with the addition of ion pair reagents like hexadecyltrimethylammonium chloride at neutral or basic pH values; and after methylation.

The separation of the underivatized compounds at acidic pH between approximately pH 2 and 4 is relatively simple. For acidification of the mobile phase, many acids and buffers can be used. For example, to separate nitrophenols, in addition to octogen and hexogen, a 0.01 mol L^{-1} sodium dihydrogen phosphate/phosphoric acid buffer (pH 3) on RP-18-columns is suitable (**Table 5**).

Table 4 Retention behaviour of neutral explosive-related compounds on different RP phases

Column	*RP-18 phase*[a] *(Eurospher 100; Knauer)*		*Nitrophenyl phase*[b] *(Cosmosil 5 NPE)*		*PGC phase*[c] *(Hypercarb; Shandon)*	
Mobile phase	*51% MeOH/49% H_2O*		*62% MeOH/38% H_2O*		*95% MeOH/5% H_2O*	
Compound	*Peak order*	*k*	*Peak order*	*k*	*Peak order*	*k*
Octogen	1	0.63	13	18.94	3	3.18
Hexogen	2	1.67	7	8.21	1	1.22
1,3,5-TNB	3	2.26	5	6.95	12	41.23
Tetryl	4	3.84	8	8.28	4	4.39
1,3-DNB	5	3.47	4	6.23	9	18.39
2,4,6-TNT	6	4.98	11	13.93	10	28.22
4-A-2,6-DNT	7	5.63	9	9.95	8	17.26
2-A-4,6-DNT	8	5.85	12	17.83	13	41.40
2,6-DNT	9	6.09	6	7.31	5	5.19
2,4-DNT	10	6.28	10	13.35	11	28.47
2-NT	11	7.60	1	4.64	2	3.00
4-NT	12	8.32	2	5.42	7	6.71
3-NT	13	8.98	3	5.62	6	5.24

[a]Column: 250 × 4 mm; 5 μm, hold-up time 1.73 min. [b]Column: 150 × 4.6 mm; 5 μm, hold-up time 1.09 min. [c]Column: 100 × 4.6 mm; 7 μm, hold-up time 1.18 min. 1 mL min^{-1}; 27°C; 10 μg mL^{-1} per component.

Table 5 Retention of nitrophenols and nitramines on RP-18 columns at pH 3

Column	*Eurospher 100 C_{18}*		*Spherisorb-ODS 2*	
Compound	*Peak order*	*k*[a]	*Peak order*	*k*[b]
Octogen	1	0.60	1	0.53
2,6-DNP	2	1.39	2	0.66
Hexogen	3	1.67	3	0.98
PA	4	2.06	4	1.07
2,4-DNP	5	2.31	5	1.13
4-NP	6	2.46	6	1.84
3-NP	7	2.76	7	2.03
2,5-DNP	8	3.22	8	2.30
3,4-DNP	9	3.25	10	2.96
4-M-2,6-DNP	10	3.38	9	2.27
3-M-2-NP	11	3.56	11	2.98
2-NP	12	3.73	12	3.10
3-M-4-NP	13	3.83	13	3.78
2-M-4,6-DNP	14	6.20	15	5.45
3,5-DNP	15	6.78	14	4.58
4-M-2-NP	16	8.37	16	7.79
5-M-2-NP	16	8.37	16	7.79

Dimension of both columns: 250 × 4 mm; 5 μm; [a]hold-up time 1.73 min; [b]hold-up time 1.59 min. 51% methanol–49% 0.01 mol L^{-1} sodium dihydrogen phosphate–phosphoric acid. buffer pH 3(v/v); 1 mL min^{-1}; 27°C; 10 μg mL^{-1} per component.

To separate nitrobenzoic acids a further decrease in pH is necessary. Good separation of these compounds in the presence of nitrophenols and nitramines can be obtained at pH 2 (addition of 0.005 mol L^{-1} sulfuric acid) and a methanol content of 47% (**Figure 7**).

Under these conditions for the determination of hexyl a gradient after 20 min to 85% methanol within 20 min is used.

Basic Compounds

The RP-18 phases based on silica are, in principle, also suitable for the determination of the metabolites diaminotoluenes, diaminonitrotoluenes and nitroanilines. For this, however, particularly inert materials with a low silanol group activity are necessary because otherwise the peaks show marked tailing and large peak widths. For example, a Eurospher column showed good properties in this respect (**Figure 8**).

We have not been able to observe an increase in separation performance with falling pH improving peak shapes, as is often described in the literature. On the other hand there is, as expected a reduction in the retention under these conditions. An increase in retention by restraining the protonation of the basic compounds at pH values > 10 is not to be recommended because of the instability of the phases used.

Alternatives to the separation of the diamino compounds, which on RP-18 phases are insignificantly retained or only poorly separated should be separated on porous polymer or PGC phases, because these phases have relatively homogeneous nonpolar surfaces and high stability over the full pH range.

Even using various mobile phases and buffer systems, these columns do not show any satisfactory separation of the compounds of interest; this is due to the low efficiency of commercial columns.

Detection

UV Detection

To detect explosive-related compounds, UV is mainly used. Aromatic nitrocompounds are UV-absorbing and can, as a rule, be sensitively detected at 254 nm (**Figure 9**). At this wavelength it is also possible to detect nitramines and aminoaromatics. In addition to sensitive detection, selectivity against matrix components and eluent impurities is reached at this wavelength, as most interferents absorb at lower wavelengths.

As a rule, nitrate esters and chlorobenzenes do not show a maximum at wavelengths higher than 200 nm. Therefore, these compounds should be detected at the lowest possible wavelength. For methanol-containing eluents the minimum practicable wavelength is around 210 nm.

The UV detector shows a high linearity for the explosive-related compounds in the concentration range of about 0.01 to 100 μg mL^{-1}.

For aromatic nitro-compounds, limits of detection (LODs) reach 5–50 ng mL^{-1} (0.1–1 ng absolutely for an injection volume of 20 μL) in the sample solution. For nitrate esters and nonnitrated chlorobenzenes the LODs are around 100–250 ng mL^{-1} (or 2–5 ng absolutely).

Using variable wavelength detectors, multichannel wavelength detectors or photodiode array detectors, it is possible to optimize the selectivities and LODs for certain purposes, by measuring at the absorption maximum or at several wavelengths in one chromatographic run. For example, hexyl can be selectively detected at high and sensitively at 420 nm.

Additionally, the photodiode array detector enables compounds to be identified with marked absorption maxima and minima (e.g. nitrophenols, nitrobenzoic acids and nitroaromatics) by means of the simultaneously recordable spectra.

Electrochemical Detection

In principle, all the nitrocompounds can be determined by means of the electrochemical detector in the reduction mode. Phenols and amino compounds can be detected in the oxidation mode.

PrepBooks

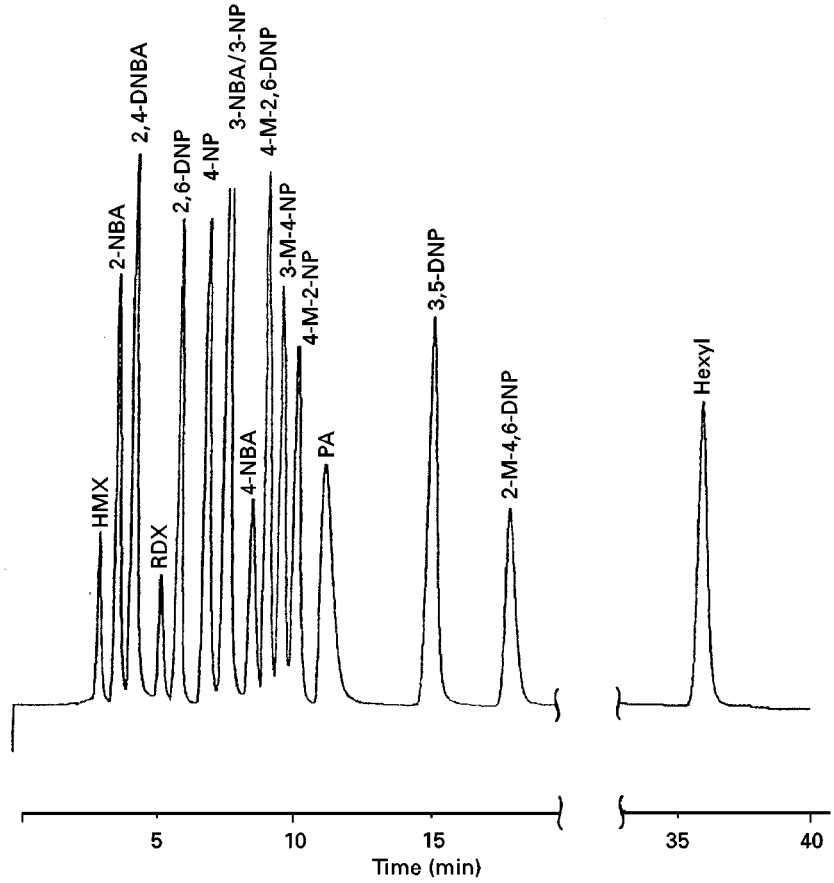


Figure 7 Chromatogram of a standard mixture of nitrophenols, nitro benzoic acids, nitramines and hexyl. Eurospher 100 RP 18 column, 5 μm, 250 × 4 mm; 47% methanol–53% 0.01 mol L^{-1} sulfuric acid (pH 2) (v/v), after 20 min linear gradient to 85% methanol within 20 min, 1.0 mL min^{-1}, 27°C; UV 254 nm. (Reproduced with permission from Lewin U *et al.* (1997) *Chromatographia* 45: 91.)

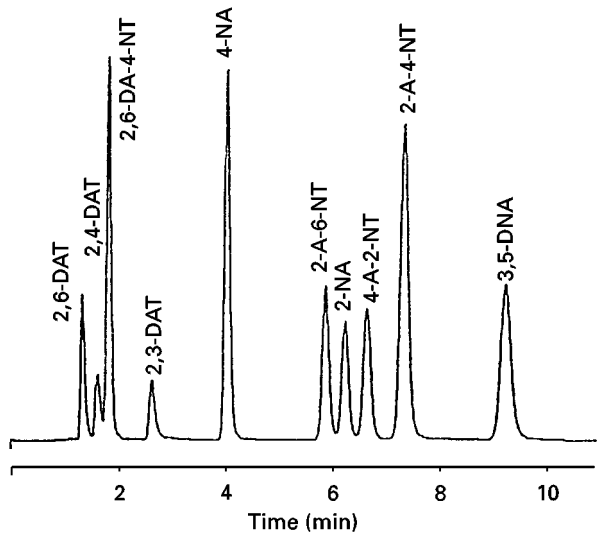


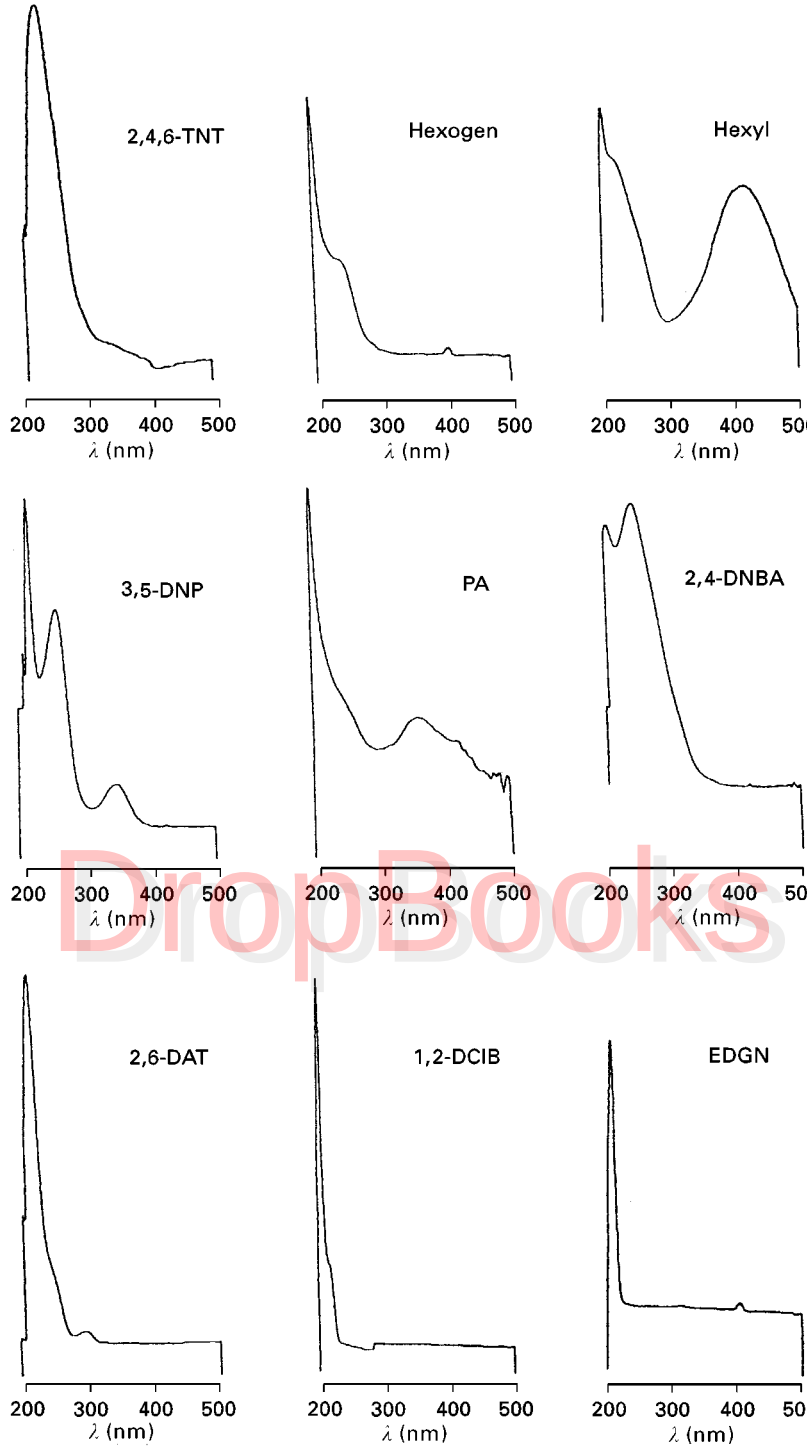
Figure 8 Chromatogram of a standard mixture of amino and diaminoaromatics Eurospher 100 RP 18 column, 5 μm, 250 × 4 mm (Knauer); 40% methanol–60% 0.01 mol L^{-1} water (v/v), 1.0 mL min^{-1}, 27°C; UV 254 nm.

The method mainly used in HPLC for electrochemical detection is amperometry: a constant optimized potential difference (working potential) is applied between the working and reference electrode, which has previously been determined from cyclovoltammograms or hydrodynamic voltammograms.

In commercially available electrochemical detectors, solid electrodes are used as they are easier to handle, although nitroaromatics can be detected sensitively with liquid mercury electrodes and in particular with the hanging mercury drop electrode.

To determine explosive-related compounds, the standard electrode material, glassy carbon, has proved its value, because it can be used over a wide potential range of about -1.3 to about $+1.3$ V. In addition, amalgamated gold electrodes or mercury film electrodes have been used for reductive detection.

The selectivity of detection can be influenced by the choice of working potential. In general, substances



DropBooks

Figure 9 Spectra of explosive-related compounds. Detected with HP 1050 variable wavelength detector in the scanning mode with 51% methanol–49% water or 0.01 mol L^{-1} phosphate buffer (pH 3) (v/v) for the acidic compounds, respectively.

whose half-wave potential is at least 150 mV larger than the working potential are not detected.

Dual cells or electrode arrays may result in an increase in the informational content of a chromatographic run or in a reduction in the limit of detection by measuring simultaneously at various potentials or at each optimal potential of the compounds.

Reduction mode Nitro compounds have very different half-wave potentials. They depend on the type,

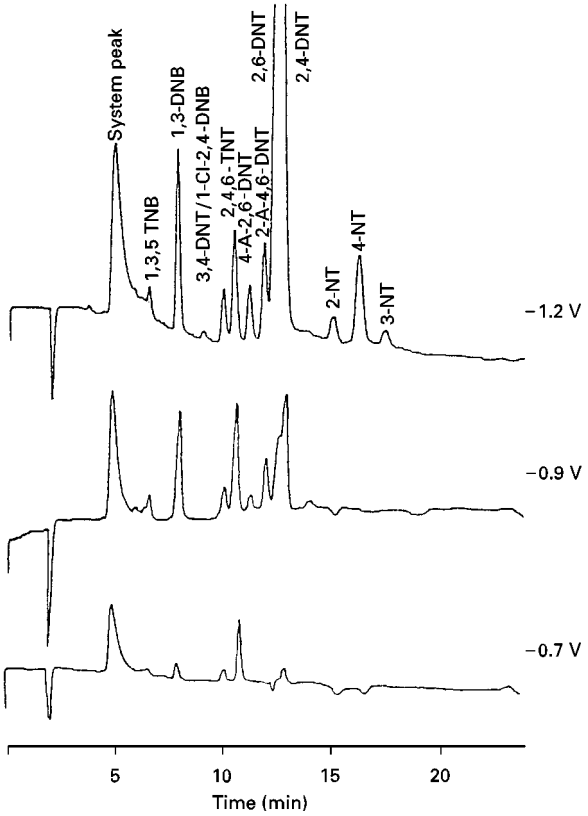


Figure 10 Electrochemical detector chromatograms of the neutral fraction of a groundwater sample from Elsnig at different potentials. Eurospher 100 RP 18 column, 5 μm, 250 × 4 mm (Knauer); 51% methanol–49% 0.01 mol L^{-1} phosphate buffer (pH 3) (v/v). (Reproduced from Lewin U *et al.* (1996) *Journal of Chromatography A* 730: 161, with permission from Elsevier Science.)

number and position of the substituents and rise, for example in the following way:

$$\text{trinitro} < \text{dinitro} < \text{mononitroaromatics} \leq \text{nitroanilines}$$

In this way a limited selective detection of certain compounds is possible (**Figure 10**). The optimum potential for the detection of all components is in perchlorate eluent (pH 5.5) at around −1.2 V.

General problems in reductive detection are caused by the difficulty of complete removal of oxygen dissolved in the mobile phase or in the sample, which is reduced at potentials of about −0.5 V and leads to greater disturbances by system peaks and high residual currents. In most cases it is not possible to reach much lower LODs than with UV detection.

Oxidation mode A great advantage of oxidative detection is that, unlike the reductive mode, oxygen has no negative influence on the detection. Like reductive detection, the aminoaromatics and nitrophenols have very different half-wave potentials, depending on the type, number and position of the substituents (**Table 6**).

For example, nitro groups will increase half-wave potentials, whereas they are reduced by amino and methyl groups, which can be utilized for selective detection.

However, the greatest selectivity advantages of anodic detection in analysing explosive-related compounds is that aminoaromatics become selective in the presence of nitroaromatics and nitramines (**Figure 11**).

Likewise, phenols and hexyl can be detected selectively, in addition to nitrobenzoic acids and nitramines (**Figure 12**).

In this way, the electrochemical detector, especially coupled with the UV detector, which is almost universal for explosive-related compounds, leads to a valuable gain in information in real samples.

Furthermore, for most nitrophenols and aminoaromatics, lower LODs are reached by anodic detection compared with UV detection. In particular, the detection limits for diamino compounds are lower by a factor of up to 100 (up to 0.05 ng mL^{-1} or 1 pg absolute) without enrichment.

Table 6 Half-wave and optimal working potentials for oxidative detection

Compound	$E_{1/2}$ *(V)*	*E opt. (V)*
2-A-4,6-DNT	1.05	> 1.20
4-A-2,6-DNT	1.05	> 1.20
2-A-3-NT	0.95	1.20
2-A-4-NT	0.95	1.20
2-A-6-NT	0.95	1.20
4-A-2-NT	0.90	1.10
2,3-DAT	0.08	0.30
2,4-DAT	0.25	0.50
2,6-DAT	0.28	0.50
2-M-4,6-DNP	1.05	1.20
4-M-2,6-DNP	1.00	1.20
2,6-DA-4-NT	0.60	0.75
2-M-3-NP	0.65	0.80
3-M-2-NP	0.65	0.80
3-M-4-NP	0.90	1.25
4-M-2-NP	0.85	1.15
4-M-3-NP	0.65	0.80
5-M-2-NP	0.85	1.15
2-NA	1.15	> 1.20
4-NA	1.05	1.20
2-NP	0.90	1.20
3-NP	0.80	1.00
4-NP	0.95	1.20
PA	> 1.20	> 1.20

Conditions: ELCD HP 1049 A with glassy carbon thin-layer working electrode and Ag/AgCl reference electrode; 51% methanol–49% 0.01 mol L^{-1} sodium dihydrogen phosphate–phosphoric acid buffer pH 3 (v/v) or sodium perchlorate solution (pH 5.5) for diaminoaromatics, respectively; 1 mL min^{-1}; 27°C.

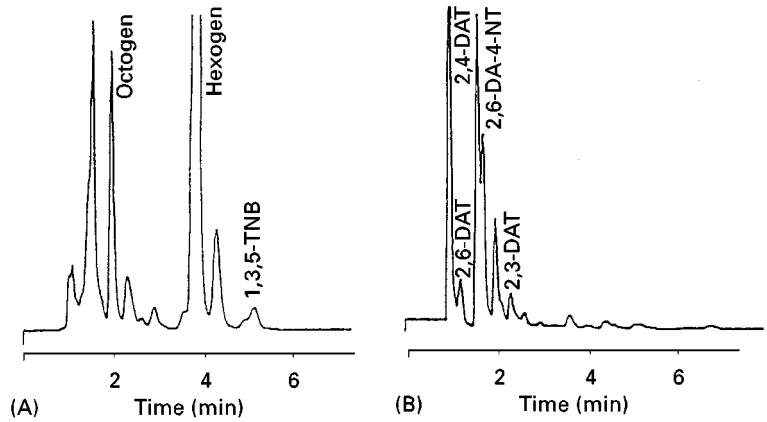


Figure 11 Chromatograms of the basic fraction of a groundwater sample from Elsnig (upper level). (A) UV (254 nm); (B) electrochemical detector (+ 0.7 V). Eurospher 100 RP 18 column, 5 μm, 250 × 4 mm; 40% methanol–60% 0.01 mol L^{-1} sodium perchlorate solution (v/v), 1.0 mL min^{-1}, 27°C. (Reproduced with permission from Lewin U *et al.* (1997) *Chromatographia* 45: 91.)

The electrochemical detector shows high linearity over a concentration range of 10^4 but, compared with UV detection, reproducibility is somewhat lower. Furthermore, there is often a decrease in the response over a long measuring period: this can be attributed to passivation of the electrode surface and requires its regeneration.

Determination of the nitroaromatics in the easier oxidation mode takes place after photolysis in a post-column reactor via the nitrite produced, which is oxidized on a glassy carbon electrode. For this, LODs of 120–250 pg have been found. The expense of the apparatus, however, is relatively high and the yield of the photolysis is very different for the various compounds.

Mass Spectrometric Detection

In the last few years the development of mass selective detectors for coupling with HPLC has made good progress. In addition to thermospray ionization (TSI),

DropBooks

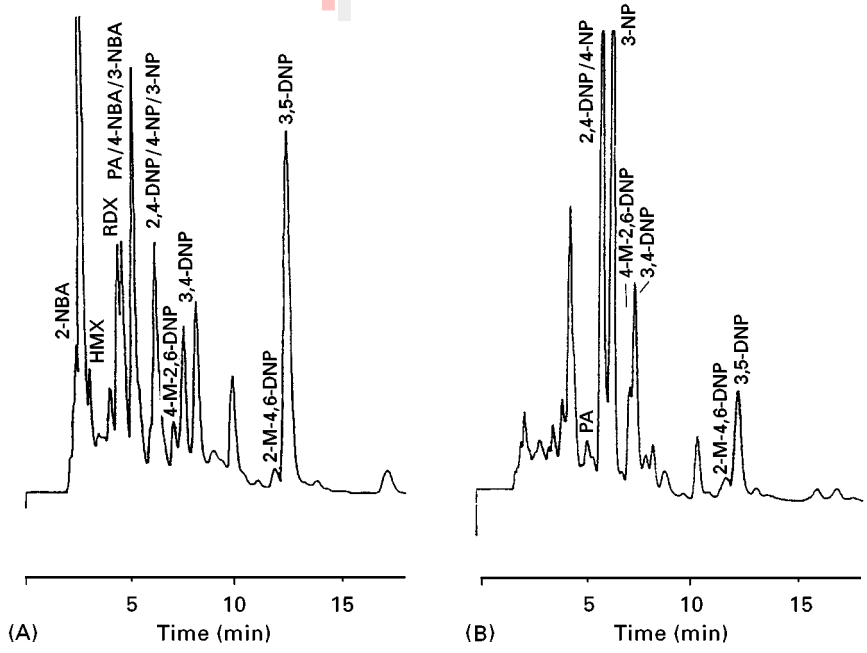


Figure 12 Chromatograms of the acidic fraction of a groundwater sample from Elsnig (lower level). (A) UV (254 nm); (B) electrochemical detector (+ 1.2 V). Eurospher 100 RP 18 column, 5 μm, 250 × 4 mm (Knauer, Berlin); 51% methanol–49% 0.01 mol L^{-1} phosphate buffer (pH 3) (v/v), after 20 min linear gradient to 80% methanol within 20 min, 1.0 mL min^{-1} (Reproduced with permission from Lewin U *et al.* (1997) *Chromatographia* 45: 91.)

the atmospheric pressure ionization (API) techniques, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are increasingly gaining in importance.

Explosives can be detected with TSI as negative ions (mostly $[M + CH_3COO]^-$ or $[M - H]^-$). Nitramines and amino compounds can be registered as positive ions (mostly $[M + NH_4]^+$). With negative ionization in the full-scan mode, LODs are in the ng mL^{-1} range. With selected ion monitoring in the filament-on negative ion mode, the LODs are a factor of 100 lower.

As a result of the electron-withdrawing effect of the nitro groups, nitroaromatics can be detected with API as negative ions $[M - H]^-$ (**Figure 13**).

The sensitivity of detection depends on the type, number and position of the substituents. It increases with the number of nitro groups and is considerably higher for nitrophenols and nitrobenzoic acids than for the corresponding neutral nitrobenzenes and nitrotoluenes.

Furthermore, sensitivity depends on the composition of the mobile phase such as the type and quantity of the organic modifier, buffer additives and pH value.

As with TSI, hexogen and octogen form clusters with acetate ions $[M + CH_3COO]^-$. A cluster formation of these compounds with ammonium ions $[M + NH_4]^+$ should, in principle, also be possible in the positive mode.

In addition to the molecular ions and molecular cluster ions, fragment ions are also formed, and elimination of oxygen or reductions and rearrangements of the nitro group are observed. Nitrophenols fragment with the loss of the hydroxyl and the nitro group. This can be used to obtain structural information. However, the assignment of position isomers is difficult, so that to investigate complex samples, carefully optimized HPLC conditions are needed and the additional use of a UV detector is an advantage.

The LODs largely depend on the particular compound and are between 0.1 and 10 μg mL^{-1} for the neutral nitroaromatics and nitramines and around 10–100 ng mL^{-1} for the nitrophenols. Reproducibility is acceptable (about 2–5%).

Nuclear Magnetic Resonance Spectroscopy

Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) is well suited for the determination of explosives because these analytes are small aromatic molecules which offer good manageable spectra; their aromatic protons appear over a relatively wide frequency range (approximately 3 ppm) because of the various substituents (nitro, carboxyl, methyl, hydroxyl and amino groups). Thus, reliable identification in very complex samples is possible by the HPLC-NMR coupling which has been developed over the last few years.

In the continuous flow mode at low flow rates (< 0.02 mL min^{-1}) and large injection volumes (approximately 400 μL), determination of explosives in the lower microgram range is possible. The reproducibility of about 2% in the upper microgram range is acceptable.

Further Detection Techniques

Fluorescence detection is not suitable for the determination of nitro compounds, as nitro groups will diminish fluorescence intensity. However amino compounds can be determined very selectively and sensitively.

As nitro compounds form nitrogen monoxide by pyrolysis, they can also be detected by a thermal energy analyser. The sensitivity depends on the substance class and is lower for nitroaromatics than for nitramines and nitrate esters. An advantage of the detector is its specificity for compounds carrying oxynitrogen functional groups. Because of the complex apparatus and the restriction to the normal-phase separation mode, the detector has not achieved wide usage.

Occasionally, reports have been published on further pre-column and post-column derivatization techniques. For example, for the amino compounds the diazotization and coupling with *N*-(1-naphtyl)-ethylenediammonium chloride into azo dyes is suitable. This reaction is also suitable for the determination of nitroaromatics after conversion with titanium(III) chloride into the amines or after photolysis and diazotization of the nitrite formed with sulfanilamide.

The electron-capture detector, widely used in gas chromatography, was not successful in practice because of incompatibility with polar mobile phases.

Conclusions

To determine the thermally unstable explosive-related compounds, the method of choice is HPLC. However, because of the limited separation performance, where there are very complex samples, HPLC determination is only possible after optimization of the method and pre-separation of the samples into different fractions.

RP-18 phases have proved their value as standard columns. In special separation problems the use of a second column is useful.

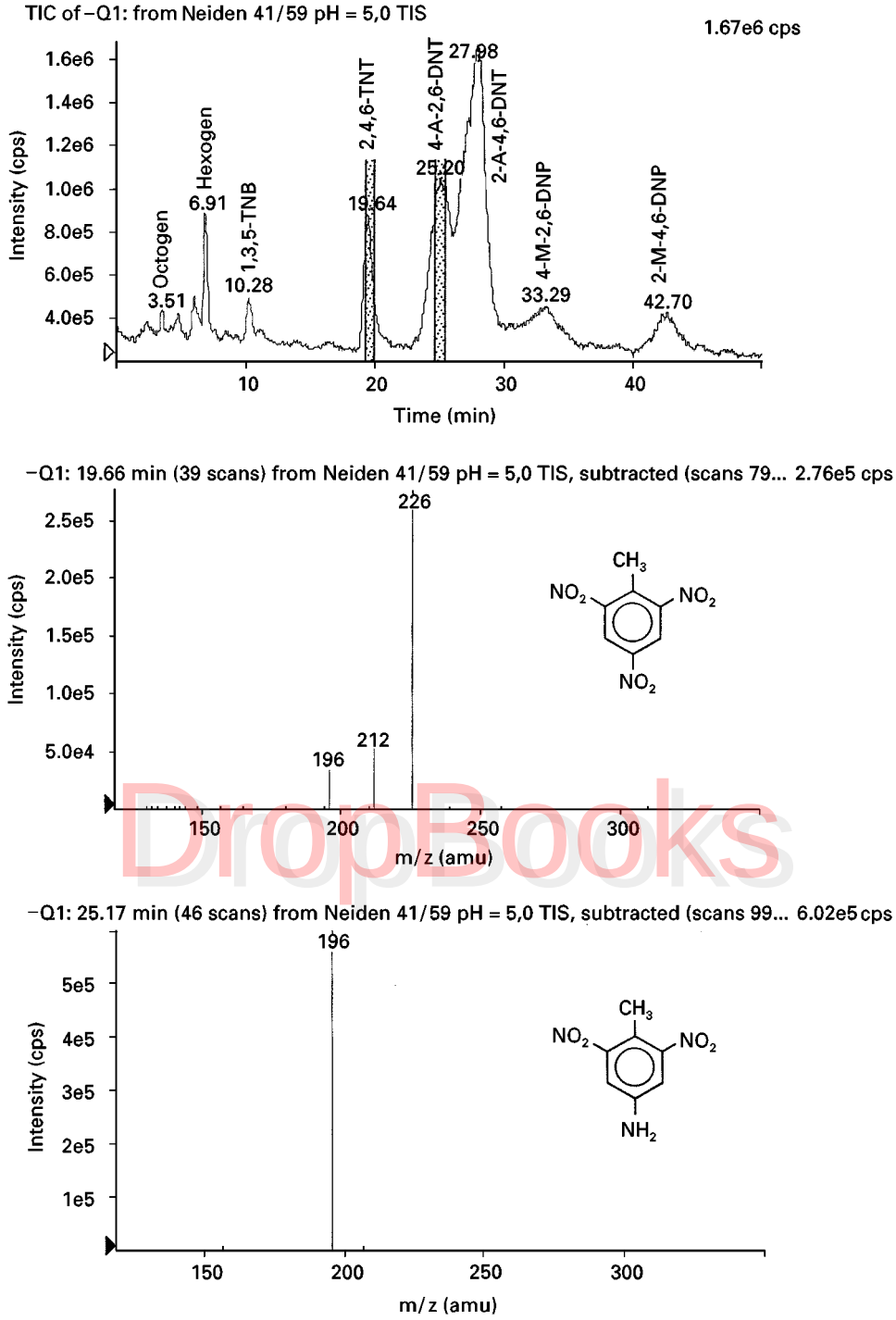


Figure 13 TIC and several mass spectra of an extract of drainage water from contaminated soil. PE-SCIEX API 100 LC/MS-System; Heated Nebulizer™ (PE), Ultrasep ES RP 18 column, 5 μm, 250 × 4 mm (Sepsev, Berlin); 41% methanol–59% water (v/v) adjusted to pH 5.0 by means of acetic acid, 20°C.

In most cases, UV detection, which is almost universal for explosive-related compounds, is used. Because of the complexity of samples, to clarify signal overlapping, and last but not least for identification, the use of selective detectors such as the electrochemical and mass spectrometric detector as well as HPLC-NMR coupling, which has been recently commercially introduced, are of great advantage.

In general, the available techniques (including sample preparation) enable explosives, their by-products and metabolites to be determined down to a range of 0.1 μg L^{-1}.

See also: **II/Chromatography: Liquid:** Detectors: Mass Spectrometry; Detectors: Ultraviolet and Visible Detection; Nuclear Magnetic Resonance Detectors. **Extraction:** Solid-Phase Extraction; Solvent Based Separation; Ultrasound Extractions. **III/Solid Phase Extraction with Cartridges.**

Further Reading

Berberich DW, Yost RA and Fetterolf DD (1988) Analysis of explosives by liquid chromatography/thermospray/mass spectrometry. *Journal of Forensic Science* 33: 946.

Bratin K, Kissinger PT, Briner RC and Bruntlett CS (1981) Determination of nitro aromatic, nitramine and nitrate ester explosive compounds in explosive mixtures and gunshot residue by liquid chromatography and reductive electrochemical detection. *Analytical Chimica Acta* 130: 295.

Fedoroff BT (ed.) (1960–83) *Encyclopedia of Explosives and Related Items*, vols 1–10. Dover: Picatinny Arsenal.

Hubball J (1992) The use of chromatography in forensic science. *Advances in Chromatography* 32: 154–172.

Jenkins TF, Leggett DC, Grant CL and Bauer CF (1986) Reverse-phase high-performance liquid chromatographic determination of nitroorganics in munitions wastewater. *Analytical Chemistry* 58: 170.

Levsen K, Preiss A and Feltes J (1995) Analysenmethoden für Explosivstoffe. In: Rippen G (ed.) *Handbuch der Umweltchemikalien* vol. 3, 31st edn, pp. 36–75. Landsberg: Ecomod.

Lewin U, Wennrich L, Efer J and Engewald W (1997) Determination of highly polar compounds in water samples around former ammunition plants. *Chromatographia* 45: 91.

Lloyd JBF (1992) HPLC of explosive materials. *Advances in Chromatography* 32: 173–261.

US Environmental Protection Agency (1992) *Method 8330, revision 1*. Washington, DC: EPA.

Yinon J and Zitrin S (1993) *Modern Methods and Applications in Analysis of Explosives*. New York: Wiley.

Thin-Layer (Planar) Chromatography

J. Bladek, Military University of Technology, Warsaw, Poland

Copyright © 2000 Academic Press

Introduction

The term explosive has two meanings. It is used for individual chemical compounds among which trinitrotoluene (TNT), 1,3,4-trinitro-1,3,5-triazacyclohexane (hexogen-RDX), 1,3,5,7-tetranitro-1,3,5,7-tetrazacyclooctane (octagen-HMX), pentaerythriol tetranitrate (pentrik-PETN) nitroglycerine (NG) and nitrocellulose (NC) are commonly known. This term is also used for mixtures of the above individual compounds and their mixtures with other, non-explosive substances; the type and amount of components in a mixture determines its properties (brisance, melting, plasticity and the like). Explosives have been classified in many ways according to different criteria. Most important is the kind of addition, the NO_2 group and type, and the velocity of the reaction involved. The first are divided into the following groups; nitro compounds containing the C-NO_2 group, nitrate esters with C-O-NO_2 group and nitramines with C-N-NO_2 group. The second criterion divides explosives into high and low explosives (HEs and LEs respectively).

The identification and quantification of the HEs or LEs are very valid and also present difficult analytical problems. These problems become evident especially during: (i) testing of environmental pollutants, (ii) forensic investigations, and (iii) checking technological processes and service conditions of munitions manufacture. From an analytical point of view, at least three groups of TLC applications in explosive investigations can be distinguished. The first of them, most often represented in the research literature, concerns qualitative (including screening methods) and quantitative analysis of explosives. The second is where TLC is applied as a clean-up technique. In this case analysis is completed by other analytical techniques. The last group of applications mainly covers the evaluation of LE stability.

Although, recently, more sophisticated methods such as thermal analyses and gas or liquid chromatography are commonly used in many laboratories, TLC is still in use. Starting from paper chromatography and improved over many years, TLC has become very effective in the analysis of explosives. Apart from high performance adsorbents, and the ability to perform separations in both normal and reversed-phase systems, the real advances are due to the development of densitometry and the spray-on technique of sampling.

Analyses of Explosives

Early TLC analysis was performed on home-made chromatographic plates and involved the separation of classical HEs such as TNT, RDX,